ORAL ARGUMENT NOT YET SCHEDULED

No. 24-1188 (lead, consolidated with No. 24-1191, No. 24-1192)

In the United States Court of Appeals For the District of Columbia Circuit

American Water Works Association and Association of Metropolitan Water Agencies,

Petitioners,

v.

United States Environmental Protection Agency, and Michael S. Regan, in his official capacity as Administrator, United States Environmental Protection Agency,

Respondents,

ON PETITION FOR REVIEW FROM FINAL RULE OF THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, 89 FED. REG. 32,532 (APR. 26,2024)

BRIEF OF PAUSTENBACH & ASSOCIATES et al. AS *AMICUS CURIAE* IN SUPPORT OF PETITIONERS

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CERTIFICATE AS TO PARTIES, RULINGS, AND RELATED CASES

Pursuant to D.C. Circuit Rule 28(a)(1), amici curiae state as follows:

A. Parties and *Amici*.

The Petitioners are American Water Works Association and Association of Metropolitan Water Agencies (No. 24-1188); National Association of Manufacturers and American Chemistry Council (No. 24-1191); and The Chemours Company FC, LLC (No. 24-1192).

The Respondents are the United States Environmental Protection Agency, Michael S. Regan (former Administrator of the United States Environmental Protection Agency), and Lee M. Zeldin, in his official capacity as Administrator, United States Environmental Protection Agency.

The *Amici* are Drs. Dennis Paustenbach, Jacob Siracusa, and Kylie McCauley (Paustenbach & Associates).

B. Rulings.

The agency action under review is a rule entitled "PFAS National Primary Drinking Water Regulation," 89 Fed. Reg. 32,532 (April 26, 2024).

C. Related Cases.

The above-captioned case (No. 24-1188) has been consolidated with two additional petitions for review, *National Ass'n of Manufacturers, et al. v. EPA, et al.*

(No. 24-1191) and *The Chemours Co. FC, LLC v. EPA, et al.* (No. 24-1192). The rule at issue has not been previously reviewed in this or any other court and there are no related cases within the meaning of Circuit Rule 28(a)(1)(C).

RULE 29 CERTIFICATES

Pursuant to Fed. R. App. P. 29(c)(5), Paustenbach and Associates state that no party or party's counsel authored this brief in whole or in part, and that no other person besides the firm contributed money that prepared or submitted the brief.

Pursuant to Circuit Rule 29(d), counsel for amicus curiae Paustenbach & Associates (the "firm") certifies that a separate brief is necessary due to the distinct scientific expertise that the firm brings to bear on this matter. The firm is owned entirely by TRC companies ("TRC"). The firm specializes in performing toxicology and risk assessment consulting related to PFAS chemicals. They apply high end traditional human health risk assessment procedures to offer opinions on numerous toxicology related issues, including PFAS, to private sector clients, the government, and others.

Although a brief has been filed by scientists in support of the Respondents, no scientific amici have submitted a brief in support of the Petitioners. Paustenbach & Associates' brief is therefore important to provide the Court with a balanced review of the important scientific issues related to this matter. This balance is important for the court to determine whether the national drinking water regulations at issue (the MCLs) are arbitrary or capricious under the Safe Drinking Water Act ("Act") The Act requires that such regulations be grounded in "the best available, peer-reviewed

science… in accordance with sound and objective scientific practice." 42 U.S.C. § 300g-1(b)(3)(A)(i).

For these reasons, counsel respectfully submits that Paustenbach & Associates should be granted leave to file its amicus curiae brief.

Dated: April 18, 2025
Richmond, VA

/s/Robert F. Redmond, Jr.
Robert F. Redmond, Jr.
Counsel for *Amicus Curiae*

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GLOSSARY OF ABBREVIATIONS

Advisory Board	EPA Science Advisory Board
DOD	Department of Defense
EPA	Respondent U.S. Environmental Protection Agency
HFPO-DA	Hexafluoropropylene oxide dimer acid (also "GenX")
Hazard Index	Index of PFAS HFPO-DA, PFBS, PFHxS, and PFNA
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MOA	Mode of action
NPDWR	National Primary Drinking Water Regulations
PFAS	Per- and Polyfluoroalkyl Substances
PFBS	Perfluorobutane Sulfonic Acid
PFHxS	Perfluorohexane Sulfonic Acid
PFNA	Perfluorononanoic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonic Acid
PPARa	Peroxisome Proliferator Activated Receptor alpha (also "PPARα protein")
RfDs	Reference doses

STATUTES AND REGULATIONS

All relevant statutory and regulatory provisions referenced in this brief are contained in the EPA's addendum.

STATEMENT OF THE CASE

The statutory and regulatory background is set forth in the American Water Works Association's Statement of the Case.

STATEMENT OF IDENTITY, INTEREST, AND AUTHORITY TO FILE

1. IDENTITY AND INTERESTS OF *AMICI CURIAE*

Amici is a consulting firm composed of leading experts on PFAS who have unique insights to offer the Court on (1) the validity of the conclusions reached by EPA, and (2) the scientific consensus about the issues addressed by the regulation in question.

Amicus Dr. Dennis Paustenbach, Ph.D., C.I.H., D.A.B.T., is a board-certified toxicologist and industrial hygienist with nearly four decades of expertise in risk assessment, toxicology, occupational health, and environmental engineering. He currently leads the Risk Sciences Division at TRC Engineering and previously served as President of Paustenbach & Associates, as well as founder and longtime leader of ChemRisk, one of the largest risk assessment consulting firms in the U.S. Over his career, he has held senior leadership roles at McLaren-Hart and Exponent and has testified in over 700 depositions and numerous trials concerning chemical exposures in various media.

A prolific scholar and educator, Dr. Paustenbach has published approximately 320 peer-reviewed articles, written more than 50 book chapters, authored leading textbooks on risk assessment, and recently edited the 7th edition of *Patty's Toxicology (a 7,000 page and 7 volume treatise).* He has co-authored several influential publications on PFAS, addressing toxicological mechanisms, risk assessment methodologies, and regulatory frameworks. Recognized for his lifetime contributions to toxicology, he received the 2024 Shubik Award from the Toxicology Forum and was named among the top 1% of environmental scientists in America by Stanford University.

Amicus Dr. Jacob Siracusa, Ph.D. is a toxicologist at TRC. He conducted his doctoral research investigating the effects of PFOS on the metabolic and immunological functions associated with alterations in the gut microbiota in mice fed a western diet. Dr. Siracusa has co-authored several publications in toxicology and public health journals. He has presented his research at several Society of Toxicology meetings. In addition, he has served as an external peer-reviewer of numerous journal articles evaluating PFAS.

Amicus Kylie McCauley is an Associate Toxicologist at TRC. Her research includes PFAS and she has worked as a consultant on several PFAS related projects. Ms. McCauley has co-authored several publications including two PFAS related publications that are in-press titled: "EPA's PFAS 2024 Drinking Water MCLs: Part

I – Analysis of Public Comments" and "EPA's PFAS 2024 Drinking Water MCLs: Part II – 15 Misconceptions About the Health Hazards and Public Health Benefits."

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) constitute a diverse class of more than 4,700 synthetic chemicals that have been widely used since the 1940s in critical applications ranging from medical devices and electronics to firefighting foams and national defense systems. PFAS are critical to the national defense of the United States and are used across the Department of Defense (DoD) in weapon platforms (e.g. fixed-wing aircraft, rotary-wing aircraft, surface ships, submarines, missiles, torpedo systems, radar systems, battle tanks, assault vehicles, and infantry carriers) and as components of plastics, O-rings, gaskets, lubricants, coolants, and fabrics.[1]

Owing to their chemical stability due to their carbon-fluorine bonds and their environmental persistence, PFAS are detectable in many environmental matrices, and have become statistically associated with certain diseases, although causation has not been established, with low-level background exposures in the general U.S. population.

[1] Department of Defense (DoD). 2023. Report on Critical Per- and Polyfluoroalkyl Substance Uses: Pursuant to Section 347 of the James M. Inhofe. National Defense Authorization Act for Fiscal Year 2023 (Public Law 117-263). Office of the Assistant Secretary of Defense for Energy, Installations, and Environment. Office of the Assistant Secretary of Defense for Industrial Base Policy, Department of Defense. August 2023. 1-27

On April 26, 2024, the U.S. Environmental Protection Agency (EPA) finalized a National Primary Drinking Water Regulation (NPDWR) that set Maximum Contaminant Levels (MCLs) at 4 parts per trillion for PFOA and PFOS, and for PFHxS, PFNA, and HFPO–DA, the MCLs were set at 10 ng/ L. A Hazard Index (HI) of 1 (unitless) was set as the Maximum Contaminant Level Goal (MCLG) and MCL for any mixture containing two or more of PFHxS, PFNA, HFPO–DA, and PFBS.[2] As discussed below, these proposed standards do not correspond to known concentrations or doses that cause adverse effects, but rather to analytical detection limits.[3] These regulatory actions mark the first enforceable federal drinking water standards for PFAS.

The EPA's derivation of reference doses (RfDs) for PFOA and PFOS was based on a limited selection of epidemiological studies that reported statistical associations between serum PFAS concentrations and various non-cancer health endpoints (e.g., immunological, developmental, cardiovascular, and hepatic). However, many of these statistical associations were weak, inconsistent, and lacked clinical significance (i.e., these statistical associations for the health endpoint did not actually lead to a measured increase in the incidence of a particular disease). Moreover, the study results were confounded by pharmacokinetic bias (i.e., the

[2] U.S. Environmental Protection Agency (EPA). 2024c. Final Rule: PFAS National Primary Drinking Water Regulation. In Federal Register, Vol 89. No. 82: April 26, 2024. 32532-32757. (EPA 2025 Fed. Reg. 89. Apr. 26, 2024)
[3] *Id*

distortion of observed exposure-outcome relationships due to differences in how individuals absorb, distribute, metabolize, or eliminate a substance), reverse causality (i.e., when the outcome influences the exposure, rather than the exposure causing the outcome), and other methodological limitations that are well documented in the scientific literature.[4,5,6,7]

Crucially, no validated mode of action (MOA) has been identified to explain how PFAS, --at environmentally relevant doses, -- could plausibly cause the array of adverse effects cited by EPA. Unlike classical toxicants that act upon identifiable target organs (e.g., the liver for carbon tetrachloride or the pleural lining for asbestos), PFAS do not seem to have a target organ, nor have researchers established any molecular initiating event relevant to human disease.[8] While it has been hypothesized that PFAS cause toxicity due to PPAR in rodents, these proteins have

[4] Verner MA, Loccisano AE, Morken NH, Yoon M, Wu H, McDougall R, Maisonet M, Marcus M, Kishi R, Miyashita C, Chen MH, Hsieh WS, Andersen ME, Clewell HJ, 3rd, Longnecker MP. 2015. Associations of Perfluoroalkyl Substances (PFAS) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK). Environmental Health Perspectives. 123(12): 1317-1324. (Verner et al. 2015)

[5] Andersen ME, Mallick P, Clewell HJ, 3rd, Yoon M, Olsen GW, Longnecker MP. 2021a. Using quantitative modeling tools to assess pharmacokinetic bias in epidemiological studies showing associations between biomarkers and health outcomes at low exposures. Environmental Research. 197: 1-13. (Andersen et al. 2021. Environ Res. 197. 1-13)

[6] Burgoon LD, Clewell HJ, Cox T, Dekant W, Dell LD, Deyo JA, Dourson ML, Gadagbui BK, Goodrum P, Green LC, Vijayavel K, Kline TR, House-Knight T, Luster MI, Manning T, Nathanail P, Pagone F, Richardson K, Severo-Peixe T, Sharma A, Smith JS, Verma N, Wright J. 2023. Range of the perfluorooctanoate (PFOA) safe dose for human health: An international collaboration. Regulatory Toxicology and Pharmacology. 145: 1-12. (Burgoon et al. 2023)

[7] Clewell H. 2024. Mode of action Criteria for selection of the critical effect and safe dose range for PFOA by the Alliance for risk assessment. Regulatory Toxicology and Pharmacology. 154: 105738. (Clewell 2024)

[8] Corton JC, Peters JM, Klaunig JE. 2018. The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Archives of Toxicology. 92(1): 83-119. (Corton et al. 2018)

much less activity in humans. The differences in responsiveness remain incompletely defined and do not explain the range of adverse effects attributed to PFAS. As stated by Parkinson et al. 2019 (pg. 326)[9]:

> "The lack of hepatomegaly and peroxisome proliferation in humans administered PPARα agonists is attributed to dose and the levels of hepatic PPARα, which are about an order of magnitude lower in humans compared with those in mice."

Despite the absence of robust toxicological or mechanistic support, EPA adopted RfDs that are at least 100- to 1000-fold lower than those issued by other credible regulatory authorities.[10] For example, EPA's RfD for PFOA (0.00003 µg/kg-day) is over 5,000 times lower than the value used by the Food Standards Australia New Zealand (FSANZ). This extraordinary divergence in regulatory values raises serious concerns about consistency, objectivity, and the scientific foundation of EPA's risk assessment.

The regulatory and economic implications of EPA's PFAS rules are profound. Several estimates project cumulative compliance and litigation costs could exceed

[9] Parkinson AJ, Ogilvie BW, Buckley FK, Parkinson O. 2019. Biotransformation of Xenobiotics. In Klaassen C. Casarett & Doull's Toxicology: The Basic Science of Poisons 9th Edition, McGraw-Hill Education.
[10] (Burgoon et al. 2023)

$1 trillion over the next decade.[11,12] Considering the impact on the U.S. economy, it is important that this major regulatory initiative be based on rigorous, reproducible scientific data, not precautionary assumptions or questionable epidemiologic data.

While the a*mici* recognize the intent of the new standards are to protect public health, we respectfully submit that the EPA's regulation of PFAS, particularly PFOA and PFOS, reflects a troubling departure from scientific norms. In contrast to the position advanced in the amicus brief filed by Drs. Birnbaum, DeWitt, Lohmann, and Schlezinger, our evaluation found no compelling basis in the toxicology or epidemiology literature to support the Agency's assertion that these regulatory limits reflect the best available science. It is our view that the EPA relied upon the precautionary principle rather than solid scientific data to support its MCL. Indeed, Birnbaum et al. (2025)[13] present very little data that clearly shows that PFAS have any toxicity at doses at least 10- to 100-fold greater than the levels to which persons are typically exposed.

[11] Wolf A. 2023. Trillions in PFAS Liabilities Threaten Corporate Bankruptcy Wave. Bloomberg Law. October 24, 2023.
[12] Magill B. 2024. Utilities Brace for Costs of Compliance With New PFAS Water Rule. Bloomberg Law. (April 11, 2024).
[13] Birnbaum L, DeWitt J, Lohmann R, Schlezinger J. 2025. Corrected Brief for Dr. Linda Birnbaum, Ph.D., Dr. Jamie DeWitt, Ph.D., Dr. Rainer Lohmann, Ph.D., and Dr. Jennifer Schlezinger, Ph.D., as *Amici Curiae* in Support of Respondents: In the United States Court of Appeals for the District of Columbia Circuit. American Water Works Association and Association of Metropolitan Water Agencies, *Petitioners* v. United States Environmental Protection Agency, and Michael S. Regan, in his official capacity as Administrator, United States Environmental Protection Agency, *Respondents*. On Petition for Review from Final Rule of the United States Environmental Protection Agency 89 Fed. Reg. 32, 532 (Apr. 26, 2024). 1-44 (Birnbaum et al. 2025)

ARGUMENT

1. THE EPA REGULATION IS NOT SUPPORTED BY THE BEST AVAILABLE SCIENCE

A. The EPA's Consideration of Expert and Public Input in PFAS Rulemaking: Differing Interpretations

Birnbaum et al. (2025, pg. 20-21)[14] argue that the U.S. Environmental Protection Agency (EPA) engaged in a transparent and scientifically robust process in formulating the National Primary Drinking Water Regulation (NPDWR) for PFAS. They emphasize the EPA's consultation with the Science Advisory Board (SAB), hosting public meetings, and soliciting stakeholder input as indicators of procedural and scientific diligence. According to their brief, the Agency's final rule reflects informed decision-making shaped by expert engagement.

We acknowledge this characterization and recognize the importance of incorporating diverse scientific viewpoints into institutional processes. However, others have argued that the EPA's engagement with experts and the public was not as comprehensive or methodologically robust as described. For example, the SAB itself raised substantive concerns during its review of the EPA's assessments for PFOA and PFOS, highlighting the lack of a predefined protocol for evaluating health effects.[15] This absence reportedly led to inconsistencies in study inclusion and

[14] *Id*

[15] Science Advisory Board (SAB). 2022. Review of EPA's Analysis to Support EPA's National Primary Drinking Water Rulemaking for PFAS Final Report. EPA-SAB-22-008. Washington, DC: U.S. Environmental Protection Agency. August. 1-147

exclusion, potentially reducing the transparency and reproducibility of the risk evaluations.[16] These issues suggest that aspects of the process may not have aligned fully with best practices and thus challenge the narrative presented in the Birnbaum et al. (2025) amicus brief.[17]

Moreover, while the EPA received over 1,600 public comments on the proposed rule, subsequent evaluations indicated that fewer than 10% were substantively addressed.[18] These comments included rigorous scientific critiques and cited more recent or methodologically sound studies than the Agency had considered. As a result, the Agency's final response emphasized less current or less robust evidence. Others have proposed that this limited responsiveness may fall short of the Safe Drinking Water Act's mandate to utilize the "best available science".[19] From this perspective, the lack of integration of high-quality public input calls into question the scientific integrity of the final maximum contaminant levels (MCLs). Indeed, we have reason to believe that nearly 1,000 written comments were not read or given serious consideration.

[16] *Id*
[17] (Birnbaum et al. 2025)
[18] Hua M, McCauley K, Brew D, Heywood J, Siracusa J, Stevens M, Paustenbach D. 2025. EPA's PFAS 2024 Drinking Water MCLs: Part I - Analysis of Public Comments [Accepted for Publication]. Critical Reviews in Toxicology. (Hua et al. 2025)
[19] (EPA 2025 Fed. Reg. 89. Apr. 26, 2024)

In addition, the EPA's application of its internal guidance, such as the *Office of Research and Development (ORD) Staff Handbook for Developing IRIS Assessments,* was often inconsistent.[20] The Agency attributed health effects to minor changes in biomarker levels that often remained within normal physiological ranges. These findings did not clearly establish causation or clinical significance, which raises questions about the scientific justification for characterizing the regulation as "health-based". This methodological departure has been noted by others, including Paustenbach et al. (2025),[21] who observed that this approach does not align with the systematic review standards outlined in the IRIS handbook.

Finally, some have suggested that external political factors may have influenced the rulemaking process. The SAB conducted its review virtually (e.g., no face-to-face meetings) and under a compressed timeline, leaving many scientific concerns unresolved. Reports have indicated that the Agency may have been pressured to finalize the rule before the 2024 election cycle.[22] Although it is not unusual for regulatory timelines to be influenced by external events, such conditions often compromise the thoroughness of scientific deliberation.

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[20] U.S. Environmental Protection Agency (EPA). 2022. ORD Staff Handbook for Developing IRIS Assessments: EPA 600/R-22/268. Washington, DC: Office of Research and Development, U.S. Environmental Protection Agency. 1-243.

[21] Paustenbach D, McCauley K, Siracusa J, Smallets S, Brew D, Stevens M, Deckard B, Hua M. 2025. EPA's PFAS 2024 drinking water MCLs: part II – 15 misconceptions about the health hazards and public health benefits [Accepted for Publication]. Critical Reviews in Toxicology. (Paustenbach et al. 2025)

[22] Elliott ED. 2024. Biden's Latest Political Payoff … With Your Money. EPA's proposed PFAS regulations will cost trillions and make water bills unaffordable for the poor. The American Spectator. (February 18, 2024).

While we acknowledge the perspective of Birnbaum et al. (2025) regarding the procedural validity of the EPA's PFAS regulation, we believe they underappreciate concerns raised by independent reviewers, including the SAB and well-respected scientists (who typically submitted comments without any financial support).[23] These concerns suggest that the scientific and legal foundations of the rule would benefit from further scrutiny, particularly in light of the statutory requirement for regulations to be based on the best available science.

B. Consideration of the Scientific and Medical Evidence for PFAS: Interpreting Limitations and Uncertainties

Contrary to the assertions presented in the amicus brief by Birnbaum et al. (2025), many experts have questioned whether the health risks attributed to PFAS at environmentally or occupationally relevant exposure levels are supported by a consistent and conclusive body of evidence.[24] Birnbaum et al. (2025) emphasized the existence of potential or theoretical hazards, drawing primarily from limited animal toxicology studies and selected epidemiological associations.[25] However, the relevance and interpretability of these studies in the context of human health risk assessment remain an area of ongoing scientific discussion. This brief examines each target organ alleged to be of concern.

[23] (Birnbaum et al. 2025)
[24] *Id*
[25] (Birnbaum et al. 2025)

It is noteworthy that one of the coauthors of the amicus brief, Dr. Birnbaum, has previously acknowledged in a peer-reviewed publication that "… researchers have not drawn a clear line between any molecular initiating event of PFAS and the pathophysiology observed in epidemiology studies".[26] This admission reflects a broader scientific recognition that, to date, there is a disconnect between mechanistic and population-level data. The associations observed in epidemiological studies, such as those linking PFAS exposure to low birth weight, certain cancers, elevated cholesterol, or liver enzyme changes, are correlational rather than causal. This distinction is critical for regulatory science, which requires a causal inference to justify risk-based standards.

Clewell (2024) discussed that the primary question in evaluating epidemiological associations is whether the observed outcomes are genuinely attributable to chemical exposure.[27] Establishing causation is essential for deriving protective and proportionate regulatory thresholds. To our knowledge, no existing study has demonstrated causality between PFAS exposure and adverse health outcomes at serum concentrations typically found in the general population. Moreover, there has been limited evidence to show that these exposures produce

[26] George AJ, Birnbaum LS. 2024. Dioxins vs. PFAS: Science and Policy Challenges. Environmental Health Perspectives. 132(8): 1-9. Page 4. (George and Birnbaum 2024)
[27] (Clewell 2024)

clinically significant effects; that is, measurable increases in disease incidence or severity.

We acknowledge that Birnbaum et al. (2025) have brought attention to studies suggesting health concerns associated with PFAS.[28] However, we believe their framing of these concerns may not fully reflect the degree of uncertainty, variability, and dose dependence that characterize the existing body of evidence. In our view, the selective emphasis on theoretical mechanisms and statistical associations, without robust confirmation of causality or clinical relevance, may overstate the health risks posed by PFAS at current exposure levels. Many studies they find convincing are those involving isolated cells where no "dose" data are available to extrapolate to humans.

As such, while they argue that precautionary regulation is warranted, others maintain that the evidentiary standard required under the Safe Drinking Water Act necessitates a more robust demonstration of risk, based on consistent, reproducible, and human-relevant data.

C. Interpreting the Strength of Recent Research on PFAS Health Effects

Birnbaum et al. (2025) cite a range of epidemiological findings linking PFAS exposure to various health endpoints, including low birth weight, cancers,

[28] (Birnbaum et al. 2025)

hypertension, elevated cholesterol, reduced vaccine response, and altered liver enzymes, to support their argument that these chemicals pose significant risks.[29] These associations have been presented as justification for strict regulatory thresholds under the EPA's final rule. However, we believe that these conclusions may overstate the strength of the underlying data, particularly regarding causality, dose relevance, and clinical significance.

Many of the associations cited are indeed statistically significant; however, they are correlational in nature. This distinction is central to toxicology and epidemiology. To illustrate the potential pitfalls of correlation-based interpretations, one might consider an analogy: while ice cream consumption is correlated with weight loss in summer months, likely due to increased physical activity and reduced appetite, this does not indicate that eating ice cream causes weight loss. Similarly, while PFAS blood concentrations in humans may co-occur with statistical differences in adverse health metrics, this does not confirm a causal relationship.

Others have argued that epidemiological studies on PFAS frequently lack supporting mechanistic or dose-response data. Regulatory decisions, particularly those grounded in quantitative risk assessment, require more than statistical association; they require consistent evidence that exposure to a chemical causes

[29] (Birnbaum et al. 2025)

harm at environmentally relevant levels. As several analyses have noted, the existing studies on PFAS do not consistently demonstrate this.[30,31,32]

We acknowledge that Birnbaum et al. (2025) underscore the importance of acting on associations that suggest potential public health concerns.[33] Nonetheless, a precautionary stance, while understandable, does not fulfill the statutory requirement that regulatory decisions be based on the "best available science".[34] Risk assessments that rely on unverified associations or modest biomarker changes (that remain within the normal physiological ranges) must be scrutinized for scientific sufficiency.

From this perspective, the EPA's decision to establish MCLs based on data that are statistically but not clinically significant reflects a precautionary approach rather than one anchored in risk-based, human-relevant evidence. While precaution is an important tool in public health policy, it should be applied within a framework that also respects evidentiary thresholds necessary for regulatory action under federal law.

[30] (Burgoon et al. 2023)
[31] (Hua et al. 2025)
[32] (Paustenbach et al. 2025)
[33] (Birnbaum et al. 2025)
[34] (EPA 2025 Fed. Reg. 89. Apr. 26, 2024)

a. Cardiovascular Risks

In their amicus brief, Birnbaum et al (2025, pg. 22-23) argue that associations between PFAS exposure and changes in serum lipids, particularly low-density lipoprotein cholesterol (LDL-C), are among the most consistent findings in human epidemiology.[35] Specifically, they stated the "… association between PFAS measured in blood and higher levels of circulating low density lipoprotein cholesterol (i.e., 'bad' cholesterol) is one of the strongest associations observed in people[36]".[37] This perspective reflects that these lipid alterations serve as early markers of cardiovascular risk and therefore justify regulatory action.

While we acknowledge that epidemiological studies have observed statistically significant increases in serum cholesterol associated with PFAS, others have noted that these changes are generally modest in magnitude and fall within normal clinical ranges. For example, the increases in LDL-C reported in many studies are in the range of 11 to 12 mg/dL, values that, although statistically detectable, are unlikely to carry meaningful health implications for the general

[35] (Birnbaum et al. 2025)
[36] Schlezinger JJ, Gokce N. 2024. Perfluoroalkyl/polyfluoroalkyl substances: links to cardiovascular disease risk. Circulation Research. 134(9): 1136-1159.
[37] (Birnbaum et al. 2025, pg. 22-23)

population.[38,39,40] Thus, while the statistical association is not disputed, some have proposed that the clinical relevance of these findings may be limited.

Birnbaum et al. (2025) also suggest that declines in PFAS serum levels are accompanied by reductions in cholesterol stating: "[w]hen PFAS levels in people decrease, so do their cholesterol levels[41,42]".[43] However, others have evaluated this hypothesis using longitudinal data and have found conflicting results. For instance, Batzella et al. (2024, pg. 7) reported that even as serum levels of PFOA, PFOS, and PFHxS declined by 62.1%, 24.4%, and 35.4%, respectively, cholesterol concentrations actually increased by 5% to 9%, including a notable rise in LDL-C.[44] Modeling analyses from the same study showed that changes in cholesterol levels were minimal, for example, a 1.40% decrease in LDL-C would lower a baseline value of 100 mg/dL to approximately 98.6 mg/dL, which falls well within normal variability.

[38] Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. American Journal of Epidemiology. 170(10): 1268-1278.
[39] Winquist A, Steenland K. 2014. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Environmental Health Perspectives. 122(12): 1299-1305.
[40] (Paustenbach et al. 2025)
[41] Fitz-Simon N, Fletcher T, Luster MI, Steenland K, Calafat AM, Kato K, Armstrong B. 2013. Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology. 24(4): 569-576.
[42] Batzella E, Rosato I, Pitter G, Da Re F, Russo F, Canova C, Fletcher T. 2024. Determinants of PFOA Serum Half-Life after End of Exposure: A Longitudinal Study on Highly Exposed Subjects in the Veneto Region. Environmental Health Perspectives. 132(2): 1-9. (Batzella et al. 2024)
[43] (Birnbaum et al. 2025, pg. 23)
[44] (Batzella et al. 2024)

Day-to-day fluctuations in LDL-C, which can be as high as 8% would likely obscure such minor shifts, suggesting that the magnitude of PFAS-related lipid effects may not be biologically significant.[45] Therefore, while the statistical association is acknowledged, its utility as a basis for regulation has been widely questioned.

Birnbaum et al. (2025, pg. 23) also reference animal studies to support their claims, stating: "[m]ice, fed an American-type diet high in cholesterol and fat and treated with moderate levels of PFOA or a PFAS mixture, develop an increase in circulating cholesterol[46,47,48]".[49] However, these studies often utilize doses that are several orders of magnitude above those experienced by the general population. One cited study involved doses equivalent to 500,000 parts per trillion (ppt), or approximately 350 times higher than PFAS serum levels measured in the 2017-2018 NHANES dataset.[50] Others have observed inconsistent effects across rodent models,

[45] Bookstein L, Gidding SS, Donovan M, Smith FA. 1990. Day-to-Day Variability of Serum Cholesterol, Triglyceride, and High-Density Lipoprotein Cholesterol Levels. Archives of Internal Medicine. 150(8): 1653-1657.
[46] Rebholz SL, Jones T, Herrick RL, Xie C, Calafat AM, Pinney SM, Woollett LA. 2016. Hypercholesterolemia with consumption of PFOA-laced Western diets is dependent on strain and sex of mice. Toxicology Reports. 3: 46-54. (Rebholz et al. 2016)
[47] Schlezinger JJ, Puckett H, Oliver J, Nielsen G, Heiger-Bernays W, Webster TF. 2020. Perfluorooctanoic acid activates multiple nuclear receptor pathways and skews expression of genes regulating cholesterol homeostasis in liver of humanized PPARα mice fed an American diet. Toxicology and Applied Pharmacology. 405: 1-14. (Schlezinger et al. 2020)
[48] Roth K, Yhang Z, Agarwal M, Liu W, Peng Z, Long Z, Birbeck J, Westrick J, Liu W, Petriello MC. 2021. Exposure to a Mixture of Legacy, Alternative, and Replacement Per- and Polyfluoroalkyl Substances (PFAS) Results in Sex-Dependent Modulation of Cholesterol Metabolism and Liver Injury. Environment International. 157: 1-20.
[49] (Birnbaum et al. 2025)
[50] Agency for Toxic Substances and Disease Registry (ATSDR). 2021. Toxicological Profile for Perfluoroalkyls. U. S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. May 2021. 1-993

and experts have cautioned that the underlying differences in lipid metabolism between rodents and humans make this extrapolation untenable.[51,52,53]

Mechanistic studies involving humanized mouse models are also referenced in their amicus brief. Birnbaum et al. (2025, pg. 24) argued that "… mice that have been genetically engineered to express the human form of PPARα respond to PFOA exposure with an increase in circulating cholesterol regardless of the diet they are fed[54]".[55] While these models are valuable for understanding receptor-level interactions, others have noted that such studies are conducted at doses which would never be observed in humans. The available data do not suggest cholesterol elevations at environmentally relevant exposure levels. These minor changes in cholesterol are a "bridge too far" to say that these changes are equal to heart disease. Proposed mechanisms, such as altered bile acid transport or suppressed cholesterol catabolism, remain speculative and have not been confirmed in human populations.[56]

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[51] Gordon SM, Li H, Zhu X, Shah AS, Lu LJ, Davidson WS. 2015. A comparison of the mouse and human lipoproteome: suitability of the mouse model for studies of human lipoproteins. Journal of Proteome Research. 14(6): 2686-2695.
[52] Andersen ME, Hagenbuch B, Apte U, Corton JC, Fletcher T, Lau C, Roth WL, Staels B, Vega GL, Clewell HJ, 3rd, Longnecker MP. 2021b. Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms. Toxicology. 459: 1-12.
[53] U.S. Environmental Protection Agency (EPA). 2023b. PFAS National Primary Drinking Water Regulation Rulemaking. In Federal Register, Vol 88, U.S. Environmental Protection Agency. No. 60: March 29, 2023. 18638-18754.
[54] (Schlezinger et al. 2020)
[55] (Birnbaum et al. 2025)
[56] Andersen et al. 2021. Environ Res. 197. 1-13.

Birnbaum et al. (2025, pg. 24) also suggest that PFAS may interfere with proteins essential for cholesterol breakdown, stating that "… PFAS can also reduce the amount of a protein that is necessary to break down cholesterol ... [57,58,59,60,61,62]".[63] However, several commenters in the public record cautioned against the use of total cholesterol as an endpoint for regulatory purposes without supporting clinical outcomes such as cardiovascular disease.[64,65] Some researchers have proposed alternative explanations, including reverse causality, whereby physiological factors such as bile acid metabolism may influence PFAS retention rather than PFAS altering lipid profiles.[66,67,68] Such interpretations challenge the directionality of the observed associations and suggest that the mechanistic link remains unresolved.

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[57] (Rebholz et al. 2016)
[58] Cui R, Li C, Wang J, Dai J. 2019. Induction of hepatic miR-34a by perfluorooctanoic acid regulates metabolism-related genes in mice. Environmental Pollution. 244: 270-278.
[59] Louisse J, Rijkers D, Stoopen G, Janssen A, Staats M, Hoogenboom R, Kersten S, Peijnenburg A. 2020. Perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorononanoic acid (PFNA) increase triglyceride levels and decrease cholesterogenic gene expression in human HepaRG liver cells. Archives of Toxicology. 94(9): 3137-3155.
[60] (Schlezinger et al. 2020)
[61] Behr AC, Kwiatkowski A, Stahlman M, Schmidt FF, Luckert C, Braeuning A, Buhrke T. 2020. Impairment of bile acid metabolism by perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in human HepaRG hepatoma cells. Archives of Toxicolgy. 94(5): 1673-1686.
[62] Murase W, Kubota A, Ikeda-Araki A, Terasaki M, Nakagawa K, Shizu R, Yoshinari K, Kojima H. 2023. Effects of perfluorooctanoic acid (PFOA) on gene expression profiles via nuclear receptors in HepaRG cells: Comparative study with in vitro transactivation assays. Toxicology. 494: 1-10.
[63] (Birnbaum et al. 2025)
[64] (Hua et al. 2025)
[65] (Paustenbach et al. 2025)
[66] Andersen et al. 2021. Environ Res. 197. 1-13.
[67] American Water Works Association (AWWA). 2023. AWWA Comments on the Proposed "PFAS National Primary Drinking Water Regulation Rulemaking" EPA-HQ-OW-2022-0114. American Water Works Association. May 30, 2023. 1-186 (AWWA 2023)
[68] (Hua et al. 2025)

While Birnbaum and colleagues cite two U.S.-based studies reporting "… a significant increase in atherosclerosis associated with PFNA, PFHxS, and PFOS[69,70,71,72]".[73] Others have emphasized the inconsistent nature of these findings. For example, a large, nested case-control study from Sweden found no elevated risk of myocardial infarction or stroke, even in individuals with high PFAS exposure.[74] The lack of temporal or dose-response consistency across studies limits the ability to draw firm conclusions about causality or generalizability.

Finally, Birnbaum et al. (2025, pg. 25) refer to studies associating PFOA and PFOS with cardiovascular mortality, claiming "… PFOA and PFOS have been associated with greater risk of death from cardiovascular disease". However, the broader literature has not confirmed such effects. Longitudinal studies in highly exposed communities have not demonstrated increased cardiovascular mortality or

[69] Osorio-Yanez C, Sanchez-Guerra M, Cardenas A, Lin PD, Hauser R, Gold DR, Kleinman KP, Hivert MF, Fleisch AF, Calafat AM, Webster TF, Horton ES, Oken E. 2021. Per- and polyfluoroalkyl substances and calcifications of the coronary and aortic arteries in adults with prediabetes: Results from the diabetes prevention program outcomes study. Environment International. 151: 1-10.
[70] Koskela A, Ducatman A, Schousboe JT, Nahhas RW, Khalil N. 2022. Perfluoroalkyl Substances and Abdominal Aortic Calcification. Journal of Occupational and Environmental Medicine. 64(4): 287-294.
[71] Biggeri E, Stoppa G, Facciolo L, Fin G, Mancini S, Manno V, Minelli G, Zamagni F, Zamboni M, Catelan D, Bucchi L. 2024. All-cause, cardiovascular disease and cancer mortality in the population of a large Italian area contaminated by perfluoroalkyl and polyfluoroalkyl substances (1980-2018). Environmental Health. 23: 1-20.
[72] Yang J, Wang T, Li K, Wang Y. 2025. Associations between per- and polyfluoroalkyl chemicals and abdominal aortic calcification in middle-aged and older adults. Journal of Advanced Research. 70: 203-222.
[73] (Birnbaum et al. 2025, pg. 24-25)
[74] Schillemans T, Donat-Vargas C, Lindh CH, de Faire U, Wolk A, Leander K, Akesson A. 2022. Per- and Polyfluoroalkyl Substances and Risk of Myocardial Infarction and Stroke: A Nested Case-Control Study in Sweden. Environmental Health Perspectives. 130(3): 1-11.

disease incidence.[75],[76],[77] Moreover, the absence of dose-response patterns and a validated mechanistic pathway further limits the regulatory utility of such findings.

In summary, we acknowledge that Birnbaum et al. (2025) present lipid alterations as one of the more consistent associations in PFAS literature.[78] However, others have raised valid concerns regarding the clinical relevance, consistency, and causality of these findings. Without evidence of meaningful health outcomes or validated mechanisms, reliance on lipid shifts alone may not meet the evidentiary threshold required for enforceable regulatory action under the Safe Drinking Water Act.

b. Cancer Risks

In their amicus brief, Birnbaum et al. (2025, pg. 25) support the EPA's classification of PFOA and PFOS as "likely to be carcinogenic to humans", citing animal bioassays, mechanistic data, and epidemiological studies as justification for this designation.[79] We acknowledge that these types of data are often considered collectively in hazard identification; however, others have raised substantive

[75] (Winquist and Steenland 2014)
[76] Steenland K, Fletcher T, Stein CR, Bartell SM, Darrow L, Lopez-Espinosa MJ, Ryan PB, Savitz DA. 2020. Review: Evolution of evidence on PFOA and health following the assessments of the C8 Science Panel. Environment International. 145: 1-12.
[77] (Schillemans et al. 2022)
[78] (Birnbaum et al. 2025)
[79] (Birnbaum et al. 2025)

concerns about the scientific relevance and human applicability of the underlying evidence.

Central to the EPA's and Birnbaum et al.'s (2025, pg. 25-26) position is the observation of tumors in rodent studies, including testicular, liver, and pancreatic tumors.[80] However, these tumors are widely understood to result from the activation of peroxisome proliferator-activated receptor alpha (PPARα), a mode of action (MOA) that has limited relevance to humans.[81,82,83,84] Numerous studies have demonstrated that human PPARα differs markedly from its rodent counterpart in terms of expression, ligand affinity, and downstream gene activation.[85,86] This suggests that these tumorigenic outcomes may not be predictive of human cancer risk. Indeed, some authorities believe rodent studies involving cancer have no relevance for predicting the human cancer health hazard.

Birnbaum et al. (2025, pg. 25) stated that "[o]ne of these studies, conducted by the U.S. National Toxicology Program, showed evidence of testicular Leydig cell,

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[80] *Id*
[81] Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA, Fenner-Crisp PA. 2003. PPARα agonist-induced rodent tumors: modes of action and human relevance. Critical Reviews in Toxicology. 33(6): 655-780. (Klaunig et al. 2003)
[82] Corton JC, Cunningham ML, Hummer BT, Lau C, Meek B, Peters JM, Popp JA, Rhomberg L, Seed J, Klaunig JE. 2014. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARα) as a case study. Critical Reviews in Toxicology. 44(1): 1-49. (Corton et al. 2014)
[83] (Corton et al. 2018)
[84] Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, Goodman J, Haber LT, Jacobs A, Klaunig JE, Lynch AM, Moggs J, Pandiri A. 2018. Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regulatory Toxicology and Pharmacology. 92: 1-7.
[85] Klaunig JE, Hocevar BA, Kamendulis LM. 2012. Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and Human Relevance. Reproductive Toxicology. 33(4): 410-418. (Klaunig et al. 2012)
[86] (Corton et al. 2018)

pancreatic acinar cell, and hepatocellular tumors in a rat chronic bioassay[87]".[88] While these findings are notable, others have argued that such tumors are hallmark responses to PPARα activation in rodents and are not observed in similarly exposed human populations. Moreover, the exposure levels required to induce these effects in animals are several orders of magnitude higher than those experienced by the general public. Regulatory conclusions based on high-dose animal bioassays, without contextualization of exposure relevance or species-specific biology, may overstate the risk to humans.

Birnbaum et al. also cited epidemiological studies that reported associations between PFOA and cancers such as testicular and kidney cancers stating: "[t]he PFOA designation was also based on epidemiological studies that report evidence of kidney and testicular cancer".[89] These studies have received considerable attention; however, other experts have pointed out that small sample sizes, lack of consistent dose-response relationships, and insufficient control for potential confounders, such as co-exposures or lifestyle factors, limit them. Indeed, as noted by Steenland and

[87] National Toxicology Program (NTP). 2023. NTP technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CASRN 335-67-1) administered in feed to Sprague Dawley (Hsd: Sprague Dawley® SD®) rats (revised). NTP TR 598. Research Triangle Park, NC: National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services. Revised February 2023. 1-173
[88] (Birnbaum et al. 2025)
[89] (Birnbaum et al. 2025)

Winquist (2021)[90] and Perez et al. (2023)[91], the available data on PFAS and cancer "remain sparse" and do not meet the key criteria for causal inference, such as the Bradford Hill criteria.

We acknowledge Birnbaum et al.'s (2025) admission that the evidence for PFOS carcinogenicity is less compelling than for PFOA.[92] They cite "[t]he evidence base for PFOS was not as strong as for PFOA" and added that "… a chronic cancer bioassay in rats was determined to be sufficiently strong to support the other observations".[93] However, the tumors observed, such as liver and pancreatic islet cell tumors occurred at exposure levels vastly exceeding environmental relevance and were associated with mechanisms not active in humans[94],[95],[96],[97],[98],[99],[100] Thus, others have argued that the scientific basis for classifying PFOS as a likely human carcinogen remains uncertain.

[90] Steenland K, Winquist A. 2021. PFAS and cancer, a scoping review of the epidemiologic evidence. Environmental Research. 194: 1-28.
[91] Perez A, Lumpkin M, Kornberg T, Schmidt A. 2023. Critical endpoints of PFOA and PFOS exposure for regulatory risk assessment in drinking water: Parameter choices impacting estimates of safe exposure levels. Regulatory Toxicology and Pharmacology. 138: 1-5.
[92] (Birnbaum et al. 2025)
[93] *Id, pg. 26*
[94] (Klaunig et al. 2003)
[95] (Klaunig et al. 2012)
[96] Mulvihill JJ. 2012. Preconception exposure to mutagens: medical and other exposures to radiation and chemicals. Journal of Community Genetics. 3(3): 205-211.
[97] (Corton et al. 2014)
[98] Steinbach TJ, Maronpot RR, Hardisty JF. 2015. Human Relevance of Rodent Leydig Cell Tumors. In Harbison RD, Bourgeois MM, Johnson GT. Hamilton & Hardy's Industrial Toxicology. Hoboken, New Jersey, John Wiley & Sons, Inc.: 1189-1196.
[99] (Corton et al. 2018)
[100] (Felter et al. 2018)

Birnbaum et al. (2025, pg. 26) further reference recent International Agency for Research on Cancer (IARC) evaluations, noting that PFOA has been classified as Group 1 (carcinogenic to humans) and PFOS as Group 2B (possibly carcinogenic).[101] We recognize the importance of IARC classification in hazard identification, but it is also important to emphasize that IARC's process does not consider real-world exposure or risk; only potential hazard (e.g., dose is not considered). Neither IARC nor EPA, in this context, incorporated quantitative exposure data when establishing these classifications.[102,103,104] As a result, others have proposed that relying on hazard-based classification from high-dose animal studies may be insufficient to support enforceable standards under a risk-based regulatory framework.

The doses required to induce tumors in animal studies are instructive in this context. PFOA-induced liver tumors, for example, occurred at oral exposures of 1.1 to 4.6 mg/kg/day, equivalent to drinking water concentrations more than 250 million times higher than the EPA's final regulatory limit of 4 ppt.[105,106] This vast margin

[101] (Birnbaum et al. 2025)
[102] Ezekiel CN, Sulyok M, Frisvad JC, Somorin YM, Warth B, Houbraken J, Samson RA, Krska R, Odebode AC. 2013. Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. International Journal of Food Microbiology. 162(3): 231-236.
[103] (EPA 2025 Fed. Reg. 89. Apr. 26, 2024)
[104] (Paustenbach et al. 2025)
[105] (NTP 2023)
[106] (Paustenbach et al. 2025)

highlights the challenges of extrapolating from high-dose bioassays to real-world human exposures.

Mechanistic data are also central to the cancer designation. Birnbaum et al. (2025) assert that PFOA and PFOS have mechanistic support for carcinogenicity,[107] yet others have noted that genotoxicity has not been observed in most studies. In fact, genotoxic responses have typically occurred only at cytotoxic doses, which lack relevance to environmental exposures.[108] In addition, tumor formation in rodent models has been predominantly associated with non-genotoxic mechanisms, such as PPARα-mediated liver hypertrophy. These are not as sensitive in humans and have limited relevance.[109]

In light of these findings, others have proposed that the mechanistic evidence cited to support the carcinogenic classification overstates the actual risk to humans at environmentally relevant doses. Regulatory frameworks such as the Safe Drinking Water Act require that enforceable limits be based on demonstrable human relevance, including dose concordance and biological plausibility. While suggestive

[107] (Birnbaum et al. 2025, pg. 25)
[108] Butenhoff JL, Kennedy GL, Jung R, Chang SC. 2014. Evaluation of perfluorooctanoate for potential genotoxicity. Toxicology Reports. 1: 252-270.
[109] Temkin AM, Hocevar BA, Andrews DQ, Naidenko OV, Kamendulis LM. 2020. Application of the Key Characteristics of Carcinogens to Per and Polyfluoroalkyl Substances. International Journal of Environmental Research and Public Health. 17: 1-30.

of hazard, we believe the current evidence does not provide sufficient weight-of-evidence for a robust risk assessment of the cancer hazard.

c. Immunotoxicity Risk

Although the EPA identified immunotoxicity --specifically reduced vaccine antibody responses,-- as a critical health endpoint in its April 2024 Final Rule, Birnbaum et al. (2025) did not address this endpoint in their amicus brief. We acknowledge the importance of vaccine efficacy as a public health concern and agree that any potential chemical interference with immune system function warrants careful consideration.[110] However, others have questioned whether the studies cited by the EPA and relied upon in the Final Rule provide sufficient evidence of clinically meaningful immunotoxic effects.

The EPA's conclusion was based primarily on three epidemiological studies: Budtz-Jorgensen and Grandjean (2018),[111] Timmermann et al. (2022),[112] and Zhang et al. (2023)[113]. These studies reported statistically significant associations between PFAS serum concentrations and lower vaccine antibody titers in children. While

[110] (Birnbaum et al. 2025)
[111] Budtz-Jorgensen E, Grandjean P. 2018. Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS One. 13(10): 1-14.
[112] Timmermann CAG, Pedersen HS, Weihe P, Bjerregaard P, Nielsen F, Heilmann C, Grandjean P. 2022. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. Environmental Research. 203: 1-7.
[113] Zhang L, Louie A, Rigutto G, Guo H, Zhao Y, Ahn S, Dahlberg S, Sholinbeck M, Smith MT. 2023. A systematic evidence map of chronic inflammation and immunosuppression related to per- and polyfluoroalkyl substance (PFAS) exposure. Environmental Research. 220: 1-16.

these findings have contributed to concern regarding PFAS-related immunomodulation, it is important to note that in all three studies, the observed antibody levels remained within clinically protective thresholds. None of the studies documented increases in infection rates, immune dysfunction, or other adverse immune outcomes.[114,115]

Others have proposed that the statistically significant decreases in antibody titers are not, in themselves, indicative of a clinically adverse effect. Under prevailing medical and regulatory definitions, immunosuppression typically requires a demonstrable loss of immune function or increased susceptibility to disease. As such, some have argued that small shifts in antibody titers, especially when those titers remain within protective ranges, do not provide sufficient justification for identifying immunotoxicity as a critical effect.

International health authorities have also weighed in on this issue. The World Health Organization (WHO), Food Standards Australia New Zealand (FSANZ), and the European Food Safety Authority (EFSA) have each concluded that antibody titer reductions, in the absence of clinically adverse immune outcomes, are insufficient

[114] World Health Organization (WHO). 2022. DRAFT: PFOS and PFOA in Drinking-water: Background Document for Development of WHO Guidelines for Drinking Water Quality. World Health Organization. September 29, 2022. 1-118 (WHO 2022)
[115] (Perez et al. 2023)

to classify PFAS as immunotoxicants.[116,117] These perspectives underscore the importance of distinguishing between statistically significant findings and outcomes that reflect true functional impairment.

The EPA also cited rodent studies, such as those by DeWitt et al. (2008)[118], in support of its immunotoxicity conclusions. However, these studies used doses hundreds to thousands of times greater than those associated with human environmental exposures. The resulting serum PFAS concentrations exceeded those seen even in highly exposed human populations by more than two orders of magnitude, raising concerns about the applicability of the findings to real-world scenarios.

Moreover, the human epidemiological studies cited by the EPA have been critiqued for methodological limitations. These include the lack of repeated immune function assessments, the absence of national baseline antibody reference values, and insufficient control for potential confounders such as socioeconomic status, nutritional factors, and co-exposures to other immunomodulatory substances.[119] Such limitations have made others question whether the data satisfy the evidentiary

[116] Food Standards Australia New Zealand (FSANZ). 2021. PFAS and Immunomodulation Review and Update. Food Standards Australia New Zealand. 1-38
[117] (WHO 2022)
[118] Dewitt JC, Copeland CB, Strynar MJ, Luebke RW. 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environmental Health Perspectives. 116(5): 644-650.
[119] (Paustenbach et al. 2025)

standards for "sound and objective scientific practices" required under the Safe Drinking Water Act.

The omission of immunotoxicity from Birnbaum et al. (2025)'s amicus brief, despite its central role in EPA's regulatory justification, may suggest that even proponents of the regulation acknowledge the relatively limited strength of the data for concern about this endpoint.[120] While this may have been an editorial choice, others have interpreted the absence as reflective of scientific uncertainty.

In conclusion, although reduced antibody responses have been cited as a basis for deriving reference doses (RfDs) and maximum contaminant levels (MCLs), others have contended that the underlying data do not demonstrate functional or clinically adverse immune outcomes. In light of these concerns, the EPA's use of immunotoxicity as a key regulatory endpoint has been viewed by some as insufficiently supported by human-relevant evidence and, therefore, not aligned with the "best available science" standard required for enforceable regulation under the Safe Drinking Water Act.

d. Developmental Risks

The EPA's 2024 Final Rule identified developmental toxicity, specifically reduced birth weight, as a critical endpoint in its derivation of regulatory values for

[120] (Birnbaum et al. 2025)

PFAS. However, Birnbaum et al. (2025) did not include a detailed discussion of this endpoint in their amicus brief, despite its central role in the Agency's justification. While this omission may reflect an editorial decision, others have observed that it raises questions regarding the strength of the evidence supporting developmental toxicity as a key regulatory driver.[121]

The EPA's designation relied primarily on three epidemiological studies to derive the reference dose for developmental toxicity. Specifically, the EPA relied on: Darrow et al. (2013), Sagiv et al. (2018), and Wikstrom et al. (2020) each of which reported modest associations between prenatal PFAS exposure and reduced birth weight or shortened gestational duration. While clinically low birth weights (i.e., < 2,500 grams) were observed in these populations, the incidence of low birth weight did not exceed the frequency expected in the general population.[122,123,124] In addition, the study authors themselves acknowledged the limited clinical relevance of the observed changes.

[121] (Birnbaum et al. 2025)
[122] Darrow LA, Stein CR, Steenland K. 2013. Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environmental Health Perspectives. 121(10): 1207-1213. (Darrow et al. 2013)
[123] Sagiv SK, Rifas-Shiman SL, Fleisch AF, Webster TF, Calafat AM, Ye X, Gillman MW, Oken E. 2018. Early-Pregnancy Plasma Concentrations of Perfluoroalkyl Substances and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? American Journal of Epidemiology. 187(4): 793-802. (Sagiv et al. 2018)
[124] Wikstrom S, Lin PI, Lindh CH, Shu H, Bornehag CG. 2020. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatric Research. 87(6): 1093-1099. (Wikstrom et al. 2020)

For example, Darrow et al. (2013) found no association between PFOS and low birth weight or preterm delivery,[125] while Sagiv et al. (2018) observed only a 1- to 3-day reduction in gestational age in males.[126] Wikstrom et al. (2020) reported a 136-gram birth weight reduction in females but no association in males.[127] These sex-specific and modest variations lacked consistency across studies and, importantly, did not indicate adverse developmental outcomes in a clinical sense. Minor reductions in birth weight or gestational age, within normal ranges, are not considered adverse under current regulatory or medical frameworks.[128,129]

Others have also noted that maternal physiology during pregnancy complicates the interpretation of PFAS levels. Physiological changes such as increased glomerular filtration rate and plasma volume expansion influence PFAS pharmacokinetics, potentially confounding exposure estimates. For instance, Verner et al. (2015)[130] and others[131,132] have proposed that these changes may contribute both to elevated PFAS levels and to minor shifts in fetal growth, suggesting that reverse causality, not PFAS, may explain some observed associations.

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[125] (Darrow et al. 2013)
[126] (Sagiv et al. 2018)
[127] (Wikstrom et al. 2020)
[128] Jain V, Singhal A. 2012. Catch up growth in low birth weight infants: striking a healthy balance. Rev Endocr Metab Disord. 13(2): 141-147.
[129] Liu X, Luo B, Peng W, Xiong F, Yang F, Wu J. 2019. Factors affecting the catch-up growth of preterm infants after discharge in China: a multicenter study based on the health belief model. Italian Journal of Pediatrics. 45: 1-6.
[130] (Verner et al. 2015)
[131] (AWWA 2023)
[132] (Paustenbach et al. 2025)

This interpretation is further supported by meta-analyses from Steenland et al. (2018)[133] and Dzierlenga et al. (2020)[134]which found that associations between PFAS and birth weight attenuation were weaker or absent when exposure was measured earlier in pregnancy, prior to these physiological changes. Thus, the biological plausibility of a causal relationship remains in question.

From a statistical standpoint, the EPA has also been criticized for emphasizing odds ratios over clinically meaningful risk estimates. High-quality studies such as Buck Louis et al. (2018)[135] and Chu et al. (2020)[136], both rated by the National Academies of Sciences, Engineering, and Medicine (NASEM) as having a low risk of bias, did not observe significant associations between PFAS and adverse birth outcomes, yet were not given substantial weight in the Agency's final assessment.[137,138] Others have argued that the selective elevation of weaker,

[133] Steenland K, Barry V, Savitz D. 2018. Serum perfluorooctanoic acid and birthweight: an updated meta-analysis with bias analysis. Epidemiology. 29(6): 765-776.
[134] Dzierlenga MW, Crawford L, Longnecker MP. 2020. Birth weight and perfluorooctane sulfonic acid: a random-effects meta-regression analysis. Environmental Epidemiology. 4(3): 1-9.
[135] Buck Louis GM, Zhai S, Smarr MM, Grewal J, Zhang C, Grantz KL, Hinkle SN, Sundaram R, Lee S, Honda M, Oh J, Kannan K. 2018. Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons. Environment International. 119: 515-526.
[136] Chu C, Zhou Y, Li QQ, Bloom MS, Lin S, Yu YJ, Chen D, Yu HY, Hu LW, Yang BY, Zeng XW, Dong GH. 2020. Are perfluorooctane sulfonate alternatives safer? New insights from a birth cohort study. Environment International. 135: 1-8.
[137] National Academies of Sciences Engineering and Medicine (NASEM). 2022. Guidance on PFAS Exposure, Testing, and Clinical Follow-Up. Washington, D.C: The National Academies Press. Page 72
[138] (Paustenbach et al. 2025)

inconsistent studies over more rigorous null findings does not reflect a balanced or comprehensive review of the evidence.[139]

Population-level trends also raise questions about the validity of the causal link between PFAS and birth weight. From 2000 to 2018, serum PFOA and PFOS concentrations among women of childbearing age in the U.S. declined by over 70% and 85%, respectively.[140] During the same period, the average birth weight decreased slightly (by approximately 30 grams), and the prevalence of low birth-weight infants increased from 7.9% to 8.3%.[141] If PFAS exposure had a substantial causal role in reduced fetal growth, these population-wide declines in exposure would be expected to correlate with improvements in birth outcomes, which has not occurred.

Finally, it is important to note that the EPA reversed its previous position without introducing new or more compelling evidence. In its 2023 assessment, the Agency concluded that the data on birth defects, fetal loss, and growth delays were

[139] (AWWA 2023); Agency for Toxic Substances and Disease Registry (ATSDR) (2024). "Fast Facts: PFAS in the U.S. Population." from https://www.atsdr.cdc.gov/pfas/data-research/facts-stats/index.html#:~:text=Blood%20levels%20of%20the%20most%20common%20PFASA&text=Since%202002%2C%20production%20and%20use,declined%20by%20more%20than%2085%25.

[140] U.S. Environmental Protection Agency (EPA). 2024b. Biomonitoring - Perfluorochemicals (PFCs). U.S. Environmental Protection Agency. https://www.epa.gov/americaschildrenenvironment/biomonitoring-perfluorochemicals-pfcs.

[141] Centers for Disease Control and Prevention (CDC). 2024. Natality Information: Live Births. Centers for Disease Control and Prevention. https://wonder.cdc.gov/Natality.html.

too limited to support regulatory action.[142] The 2024 rule, however, cited the same epidemiological studies, and several high-dose rodent bioassays that reported common, non-specific developmental effects (e.g., delayed eye opening and lower pup weight.[143,144] These effects occurred at exposure levels thousands of times greater than environmental relevance and are widely acknowledged to have limited predictive validity for human developmental outcomes.[145,146]

For instance, developmental effects were observed at serum concentrations of approximately 2,300 ng/mL [2,300,000 ppt] in mice, roughly 1,642 times higher than the median serum concentrations in the general U.S. population in 2018.[147,148] This large margin suggests that the relevance of such findings to human populations is quite limited.

In summary, while Birnbaum et al. (2025) did not focus on developmental toxicity in their brief, others have proposed that the data underlying the EPA's

[142] U.S. Environmental Protection Agency (EPA). 2023a. Economic Analysis for the Proposed Per- and Polyfluoroalkyl Substances National Primary Drinking Water Regulation. Office of Water. Office of Ground Water and Drinking Water. Standards and Risk Management Division. Washington DC. EPA. 1-374.
[143] Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, Strynar MJ. 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicological Sciences. 90(2): 510-518.
[144] U.S. Environmental Protection Agency (EPA). 2024d. FINAL: Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts: EPA Document No. 815R24006. Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division. April 2024. 1-556
[145] Nyitray M, Szaszovsky E, Druga A. 1980. Clofibrate and the development of rats. Archives of Toxicolgy. 4: 463-465.
[146] Ao J, Yuan T, Xia H, Ma Y, Shen Z, Shi R, Tian Y, Zhang J, Ding W, Gao L, Zhao X, Yu X. 2019. Characteristic and human exposure risk assessment of per- and polyfluoroalkyl substances: A study based on indoor dust and drinking water in China. Environmental Pollution. 254(Pt A): 1-9.
[147] Post GB, Cohn PD, Cooper KR. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. Environmental Research. 116: 93-117.
[148] (ATSDR 2021)

conclusions for this endpoint are limited in consistency, clinical significance, and biological plausibility.[149] The available studies report modest associations within normal clinical ranges and may be confounded by physiological or statistical artifacts. Consequently, we believe that findings of reduced birth weight do not meet the evidentiary threshold for defining a critical effect under the Safe Drinking Water Act and that use of such findings as a basis for RfDs and MCL derivation may not reflect the best available science.

e. Effects on the Liver

The EPA's 2024 Final Rule identified liver toxicity, specifically elevations in alanine aminotransferase (ALT), as a sensitive endpoint in deriving RfDs for both PFOA and PFOS.[150] Birnbaum et al. (2025), however, did not include a substantive discussion of liver effects in their amicus brief. While this may reflect the prioritization of other endpoints, this omission is notable given the centrality of hepatic endpoints in the EPA's risk assessment.[151]

The Agency's conclusion for deriving RfDs for hepatotoxicity was based primarily on three epidemiological studies: Gallo et al. (2012), Darrow et al. (2016), and Nian et al. (2019), all of which reported statistically significant associations between PFAS serum concentrations and ALT levels. However, these levels were

[149] (Birnbaum et al. 2025)
[150] (EPA 2024 FINAL: Human Health Toxicity Assessment for PFOA. 815R24006. April 2024)
[151] (Birnbaum et al. 2025)

consistent with the variation in ALT levels expected in the general population, and none of the studies documented liver disease, hepatic dysfunction, or histopathological changes.[152,153,154] For this reason, others have proposed that these associations may not reflect adverse effects in the traditional toxicological or clinical sense.

ALT is a nonspecific enzyme influenced by numerous factors unrelated to chemical exposure, including body mass index, physical activity, metabolic health, and acute inflammation.[155] In clinical toxicology, mild increases in ALT, particularly in isolation, are not generally considered indicative of liver injury unless they exceed 3 to 5 times the upper limit of normal (ULN) and are sustained over time or accompanied by elevations in other biomarkers such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, or reduced albumin.[156,157] The EPA-cited studies did not demonstrate such accompanying changes.

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[152] Gallo V, Leonardi G, Genser B, Lopez-Espinosa MJ, Frisbee SJ, Karlsson L, Ducatman AM, Fletcher T. 2012. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environmental Health Perspectives. 120(5): 655-660.
[153] Darrow LA, Groth AC, Winquist A, Shin HM, Bartell SM, Steenland K. 2016. Modeled Perfluorooctanoic Acid (PFOA) Exposure and Liver Function in a Mid-Ohio Valley Community. Environmental Health Perspectives. 124(8): 1227-1233.
[154] Nian M, Li Q-Q, Bloom M, Qian ZM, Syberg KM, Vaughn MG, Wang S-Q, Wei Q, Zeeshan M, Gurram N. 2019. Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: Isomers of C8 Health Project in China. Environmental Research. 172: 81-88.
[155] Tiller NB, Stringer WW. 2023. Exercise-induced increases in "liver function tests" in a healthy adult male: Is there a knowledge gap in primary care? Journal of Family Medicine and Primary Care. 12(1): 177-180.
[156] Bethea ED, Pratt DS. 2022. Evaluation of Liver Function. In Loscalzo J, Kasper DL, Longo DL, Fauci AS, Hauser S, Jameson JL. Harrison's Principles of Internal Medicine 21st Edition, McGraw Hill.
[157] Helsper C, van Essen G, Frijling BD, de Wit NJ. 2012. Follow-up of mild alanine aminotransferase elevation identifies hidden hepatitis C in primary care. British Journal of General Practice. 62(596): e212-e216.

However, the evidentiary strength of these associations has been questioned due to methodological limitations inherent in the cited studies. Gallo et al. (2012)[158] and Nian et al. (2019)[159] employed cross-sectional analyses, which are inherently limited in their ability to establish causality or temporal direction. Darrow et al. (2016)[160], which utilized a more refined exposure model, found no association with liver disease diagnosis, hepatic fibrosis, or other clinical endpoints. Thus, while the reported ALT increases may be statistically significant, others have argued that these increases lack toxicological or clinical relevance.[161,162]

It is also important to consider the standard diagnostic criteria for hepatotoxicity. According to Klaunig and Wang (2019)[163], liver injury requires consistent biomarker changes across time and often confirmation through histological or imaging evidence. The studies cited by EPA do not meet these diagnostic thresholds. As a result, some have argued that interpreting minor ALT elevations as evidence of liver toxicity may overstate the health implications of PFAS exposure.[164,165,166]

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[158] (Gallo et al. 2012)
[159] (Nian et al. 2019)
[160] (Darrow et al. 2016)
[161] C8 Science Panel. 2012. Probable Link Evaluation for Liver Diseases. C8 Science Panel. October 29, 2012. 1-9
[162] (Steenland et al. 2020)
[163] Klaunig JE, Wang Z. 2019. Chemical Carcinogenesis. In Klaassen CD. Casarett & Doull's Toxicology: The Basic Science of Poisons 9th Edition, McGraw Hill. 433-496.
[164] (Hua et al. 2025)
[165] (Tiller and Stringer 2023)
[166] (Bethea and Pratt 2022)

In addition, others have pointed to clinical trial data that challenge the EPA's assumptions. Convertino et al. (2018) conducted a pharmacokinetic study in which human volunteers ingested ammonium perfluorooctanoate (APFO) at doses ranging from 50 to 1,200 mg/kg, levels roughly 480,000 to 11.5 million times higher than the mean PFOA serum concentrations in the U.S. population (1.47 ng/mL).[167] Despite these extraordinarily high doses, the study found no significant changes in liver enzyme levels, including ALT, AST, or other systemic indicators of hepatic function. These findings suggest that low-level PFAS exposure may not result in clinically meaningful liver effects and call into question the causal significance of small biomarker changes observed in observational studies.

The EPA's reliance on rodent liver studies has also drawn criticism. The liver effects seen in rodents typically occur at high doses and are mediated through PPARα, a pathway known to be far more sensitive towards activation in rodents than in humans.[168,169,170] Differences in PPARα expression, receptor sensitivity, and downstream gene activation have been well-documented and widely acknowledged

[167] Convertino M, Church TR, Olsen GW, Liu Y, Doyle E, Elcombe CR, Barnett AL, Samuel LM, MacPherson IR, Evans TRJ. 2018. Stochastic Pharmacokinetic-Pharmacodynamic Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate (PFOA). Toxicological Sciences. 163(1): 293-306.
[168] (Klaunig and Wang 2019)
[169] (Corton et al. 2014)
[170] EFSA Panel on Contaminants in the Food Chain, Knutsen HK, Alexander J, Barregard L, Bignami M, Bruschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot AC, Vleminckx C, Vollmer G, Wallace H, Bodin L, Cravedi JP, Halldorsson TI, Haug LS, Johansson N, van Loveren H, Gergelova P, Mackay K, Levorato S, van Manen M, Schwerdtle T. 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal. 16(12): 1-284.

as limiting factors in extrapolating rodent liver outcomes to human health.[171,172] As stated by Klaunig and Wang (2019, pg. 473) in a classic textbook:[173]

> "Generally, the MTD [maximum tolerable dose] is used to set the high dose in a chronic [2-year animal bioassay] study. Many investigators have questioned the use of the MTD as the upper dose level, as it is recognized that the doses selected represent those that are considered unrealistically high for human exposure. Pharmacokinetics and metabolism at high doses are frequently unrepresentative of those at lower doses … a general relationship between toxicity and carcinogenicity cannot be drawn for all classes of chemicals."

As a result, others have concluded that liver toxicity, as characterized by minor ALT elevations or rodent-specific effects, does not constitute a robust or human-relevant basis for regulation. The use of such endpoints in the derivation of RfDs and enforceable drinking water standards may not align with the scientific and legal standards required under the Safe Drinking Water Act.

In summary, although the EPA identified liver effects as a key component of its regulatory rationale, others have proposed that the evidence supporting this

[171] (Felter et al. 2018)
[172] (Corton et al. 2018)
[173] (Klaunig and Wang 2019)

endpoint lacks both clinical and toxicological significance. The studies cited do not document liver disease, functional impairment, or histopathological change. The omission of this endpoint from the Birnbaum et al. (2025) amicus brief, despite its centrality to the regulation, may reflect its evidentiary limitations.[174] Regulatory decisions of such magnitude should be grounded in human-relevant endpoints with demonstrated clinical impact, and criteria that, in the case of liver toxicity, may not be sufficiently met.

2. Rodent PPARα Activation is Not Predictive of Human Health Risk: Scientific Constraints in Extrapolating Animal Mechanisms to Humans

In their amicus brief, Birnbaum et al. (2025, pg. 27) argue that toxicological effects observed in rodent studies, particularly those involving activation of the nuclear receptor PPARα, have relevance to human health and support regulatory actions on PFAS.[175] While we acknowledge that rodent bioassays play a foundational role in hazard identification, others have pointed out that there are significant interspecies differences in receptor function, sensitivity, and downstream signaling that limit the extrapolation of rodent PPARα-mediated effects to humans.

[174] (Birnbaum et al. 2025)
[175] *Id*

This position is supported by a broad scientific consensus developed over the past two decades.[176,177,178,179,180,181,182] These studies emphasize that while both rodents and humans express PPARα, the receptor behaves quite differently across species, particularly with respect to liver proliferation and tumor formation. Therefore, while the receptor's role in lipid metabolism is evolutionarily conserved, the consequences of its activation are not uniformly predictive across species.

In addition, the interpretation advanced by Birnbaum et al. (2025) does not sufficiently account for the fundamental toxicological principle that "the dose makes the poison."[183] Indeed, the dose required to activate PPARα in humans is substantially higher than in rodents. Wolf et al. (2008) demonstrated that the lowest-observed effect concentration (LOEC) for PFOA in COS-1 cells transfected with human PPARα was 10 µM (or 4.3 µg/mL), whereas the LOEC for rodent PPARα

[176] (EPA 2024 FINAL: Human Health Toxicity Assessment for PFOA. 815R24006. April 2024)
[177] (Klaunig et al. 2013)
[178] (Corton et al. 2014)
[179] (Felter et al. 2018)
[180] Chappell GA, Thompson CM, Wolf JC, Cullen JM, Klaunig JE, Haws LC. 2020. Assessment of the Mode of Action Underlying the Effects of GenX in Mouse Liver and Implications for Assessing Human Health Risks. Toxicologic Pathology. 48(3): 494-508.
[181] Heintz MM, Haws LC, Klaunig JE, Cullen JM, Thompson CM. 2023. Assessment of the mode of action underlying development of liver lesions in mice following oral exposure to HFPO-DA and relevance to humans. Toxicological Sciences. 192(1): 15-29.
[182] Li X, Wang Z, Wu Q, Klaunig JE. 2024. Evaluating the mode of action of perfluorooctanoic acid-induced liver tumors in male Sprague-Dawley rats using a toxicogenomic approach. Journal of Environmental Science and Health, Part C. 42(3): 189-213.
[183] (Birnbaum et al. 2025)

was 1 µM (or 0.43 µg/mL).[184] This tenfold difference in receptor sensitivity is further supported by molecular expression data. Klaunig et al. (2012, pg. 413) noted:[185]

> "While the liver contains PPARα, of particular relevance is that (1) approximately 10-fold less mRNA for PPARα is found in the human liver compared with rodents …"

And, similarly, Corton et al. (2014, pg. 33) reported:[186]

> "In seven lysates from individual human livers in which PPARα could be detected by the assay, the amounts ~10-fold lower than those detected in the livers of CD-1 or BALB/cByJ mice."

This differential expression and receptor sensitivity have critical implications for interpreting the relevance of rodent data. Moreover, blood PFAS concentrations in the general U.S. population, according to the 2017-2018 NHANES dataset, are far below these thresholds, typically 1.4, 4.3, and 1.1 µg/L (or ng/mL) for PFOA, PFOS, and PFHxS respectively.[187] These concentrations are roughly 300-fold lower than the LOEC for rodent PPARα activation and over 3,000-fold lower than the dose required to activate human PPARα.

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[184] Wolf CJ, Takacs ML, Schmid JE, Lau C, Abbott BD. 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicological Sciences. 106(1): 162-171.
[185] (Klaunig et al. 2012)
[186] (Corton et al. 2014)
[187] (ATSDR 2021)

While Birnbaum et al. (2025) correctly noted that PPARα is present in human liver tissues and plays a role in lipid regulation by stating: "… PPARα is highly present in human livers, which makes it an important target for drugs used to lower serum triglyceride levels … [188]"[189], others have emphasized that its downstream effects differ markedly from those observed in rodent models.[190,191,192] For instance, peroxisome proliferation, hepatocellular hypertrophy, and liver tumor formation are well-established in rodents following PPARα activation but are not replicated in humans.[193] These physiological differences challenge the assumption that rodent liver outcomes are directly applicable to human risk assessments.

Birnbaum et al. (2025, pg. 27) also reference genetic polymorphisms in human PPARα, stating: "… human PPARα is polymorphic … People who have a mutant form of PPARα have a high blood triglyceride and cholesterol level[194]".[195] While genetic variability in receptor function is a well-acknowledged phenomenon, others have noted that there is no current evidence linking PPARα polymorphisms

[188] Kersten S, Stienstra R. 2017. The role and regulation of the peroxisome proliferator activated receptor alpha in human liver. Biochimie. 136: 75-84.
[189] (Birnbaum et al. 2025)
[190] Goodrum PE, Anderson JK, Luz AL, Ansell GK. 2021. Application of a Framework for Grouping and Mixtures Toxicity Assessment of PFAS: A Closer Examination of Dose-Additivity Approaches. Toxicological Sciences. 179(2): 262-278.
[191] (Corton et al. 2018)
[192] (Felter et al. 2018)
[193] (Klaunig et al. 2003)
[194] Yong EL, Li J, Liu MH. 2008. Single gene contributions: genetic variants of peroxisome proliferator-activated receptor (isoforms alpha, beta/delta and gamma) and mechanisms of dyslipidemias. Current Opinion in Lipidology. 19(2): 106-112.
[195] (Birnbaum et al. 2025, pg. 27)

to increased sensitivity to PFAS toxicity at environmentally relevant doses.[196,197] Thus, invoking genetic heterogeneity without health outcome data does not strengthen the argument for human relevance.

We also noted that Birnbaum et al. (2025) acknowledged an important limitation that "[a]ctivation of PPARα does cause cancer in rodents, but it has not been proven to cause cancer in people".[198] This concession is critical, as it underscores the mechanistic divide between species. Numerous studies, including those involving PPARα-knockout mice, have confirmed that tumor formation following PFOA exposure is receptor-dependent and rodent-specific.[199,200] Without corroborating epidemiological or clinical evidence, mechanistic hypotheses alone, especially those not demonstrated in human tissues, offer limited regulatory utility.

They further asserted that "… peroxisome proliferation can occur in human livers" but also conceded that "it is not thought to lead to liver tumors or liver cancer in humans[201]".[202] Others have noted that peroxisome proliferation is often an adaptive response in human tissues and lacks inherent toxicity. Without associated

[196] (EPA 2024 FINAL: Human Health Toxicity Assessment for PFOA. 815R24006. April 2024)
[197] (ATSDR 2021)
[198] (Birnbaum et al. 2025, pg. 27)
[199] (Goodrum et al. 2021)
[200] (Corton et al. 2014)
[201] (Corton et al. 2018)
[202] (Birnbaum et al. 2025, pg. 28)

pathology, the presence of such cellular changes does not satisfy toxicological criteria for adversity.[203,204] As mentioned by Klaunig and Wang (2019, pg. 473):[205]

> "At such high doses, the pharmacokinetics and metabolic responses in test animals can differ significantly from those observed at lower, environmentally relevant doses."

The effects seen in rodent liver tissue following high-dose PFAS exposure cannot be generalized to human health outcomes. Thus, without evidence of pathology, peroxisome proliferation in humans lacks toxicological or regulatory relevance.

Similarly, Birnbaum et al. (2025) stated: "… PPARα is known to control multiple other biological pathways in the human liver similarly to rodents[206]".[207] While such models can provide mechanistic insights, others have cautioned that they are artificial constructs; typically with exposure doses far exceeding environmental relevance.[208] These models do not replicate the full complexity of human physiology and cannot substitute for empirical data from human populations.[209,210,211]

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[203] (Corton et al. 2018)
[204] (Klaunig et al. 2003)
[205] (Klaunig et al. 2019)
[206] Rakhshandehroo M, Hooiveld G, Muller M, Kersten S. 2009. Comparative analysis of gene regulation by the transcription factor PPARalpha between mouse and human. PLoS One. 4(8): 1-13.
[207] (Birnbaum et al. 2025, pg. 25)
[208] (Paustenbach et al. 2025)
[209] (EPA 2024 FINAL: Human Health Toxicity Assessment for PFOA. 815R24006. April 2024)
[210] (ATSDR 2021)
[211] (Steenland et al. 2020)

Finally, Birnbaum et al. (2025) reference their own research (Nielsen et al., 2022), which used a luciferase reporter assay in COS-7 cells to demonstrate PFAS-induced PPARα activation at micromolar concentrations.[212] While this confirms ligand binding, the authors did not assess downstream gene expression or cellular outcomes. Others have emphasized that binding alone does not constitute a complete MOA, especially in the absence of evidence for subsequent toxic effects at relevant doses. Corton et al. (2014) outlined that the PPARα-mediated tumor pathway includes multiple key events beyond receptor binding, including clonal expansion, preneoplastic foci formation, and tumor development. These later events are not well established in humans.[213]

Therefore, Birnbaum et al. (2025) selectively emphasize mechanistic findings in support of their conclusions while overlooking the decades of data demonstrating that the PPARα pathway, though biologically active, is not predictive of human carcinogenicity or liver toxicity.[214] The use of high-dose animal or *in vitro* data in the absence of corresponding human outcomes has limited utility for regulatory decision-making under the Safe Drinking Water Act.

[212] Nielsen G, Heiger-Bernays WJ, Schlezinger JJ, Webster TF. 2022. Predicting the effects of per- and polyfluoroalkyl substance mixtures on peroxisome proliferator-activated receptor alpha activity in vitro. Toxicology. 465: 1-14.
[213] (Corton et al. 2014)
[214] (Birnbaum et al. 2025)

In summary, while we acknowledge that Birnbaum et al. (2025) seek to demonstrate the human relevance of rodent PPARα activation, a large body of scientific evidence indicates that the downstream pathways are fundamentally rodent-specific.[215] Differences in receptor activation thresholds, biological responses, and the absence of supporting epidemiological data suggest that PPARα-mediated effects are not an appropriate basis for deriving regulatory limits for PFAS in humans.

3. The Hazard Index Approach to PFAS Mixtures is Not Scientifically Supported and Lacks a Human-Relevant Mechanistic Basis

In their amicus brief, Birnbaum et al. (2025) argue that the U.S. EPA's use of a hazard index (HI) to regulate PFHxS, PFNA, PFBS, and HFPO-DA (GenX) is scientifically appropriate.[216] They support this position by referencing studies that report dose-additive effects and suggest that PFAS mixtures pose cumulative health risks. While we recognize that cumulative risk assessment can be a valuable regulatory tool under certain conditions, others have raised concerns that the scientific basis for applying the HI approach to PFAS mixtures, (particularly in the context of these four compounds), is lacking in mechanistic coherence, human relevance, and empirical support.

[215] *Id*
[216] (Birnbaum et al. 2025)

A. Scientific Evidence Does Not Support Additive Health Effects from PFAS Mixtures or the Use of a Cumulative Hazard Index

a. Lack of Mechanistic Sensitivity and Divergent Toxicity Profiles Undermine Claims of PFAS Dose Additivity

Birnbaum et al. (2025) stated: "The EPA's choice to use the Hazard Index to assess the cumulative effect of exposure to PFAS is scientifically appropriate because scientific studies show that exposure to multiple PFAS has a cumulative effect".[217] However, others have pointed out that co-occurrence of PFAS in the environment does not, in itself, imply additive toxicity, particularly when the compounds differ in their pharmacokinetics, MOAs, and biological targets.[218],[219],[220]

The EPA's own 2023 white paper cautioned against assuming additive effects in the absence of a shared MOA or clear biological concordance. Yet, the HI framework groups PFHxS, PFNA, PFBS, and GenX are based largely on overlapping health outcomes rather than validated mechanistic pathways. Others have argued that this approach may conflict with long-standing principles of mixture toxicology, which emphasize the need for a common mechanism of action, similar

[217] *Id*, pg. 36
[218] U.S. Environmental Protection Agency (EPA). 2024a. Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS. 815R24004. U.S. Environmental Protection Agency.
[219] U.S. Environmental Protection Agency (EPA). 2023c. Advances in Dose Addition for Chemical Mixtures: A White Paper. Washington DC: U.S. Environmental Protection Agency. 1-169
[220] (Goodrum et al. 2021)

potency, and parallel dose-response curves when applying dose-additive models.[221,222]

Birnbaum et al. (2025) asserted that "[m]ulitple studies confirm that the cumulative exposure to multiple PFAS has adverse health effects".[223] While we acknowledge the presence of shared outcomes, such as antibody titer reduction or liver enzyme shifts, others have suggested that these endpoints may not reflect mechanistic similarity.[224] For example, PPARα activation has been proposed as a common pathway, yet this mechanism is widely considered to have limited relevance in humans at environmental exposure levels.[225,226,227] Therefore, relying on endpoint similarity in the absence of biological equivalence may lead to mischaracterization of health risks and regulatory overreach.

[221] Teuschler LK. 2007. Deciding which chemical mixtures risk assessment methods work best for what mixtures. Toxicology and Applied Pharmacology. 223(2): 139-147.
[222] Anderson JK, Brecher RW, Cousins IT, DeWitt J, Fiedler H, Kannan K, Kirman CR, Lipscomb J, Priestly B, Schoeny R, Seed J, Verner M, Hays SM. 2022. Grouping of PFAS for human health risk assessment: Findings from an independent panel of experts. Regulatory Toxicology and Pharmacology. 134: 1-9.
[223] (Birnbaum et al. 2025, pg. 36)
[224] (Corton et al. 2014)
[225] *Id*
[226] U.S. Environmental Protection Agency (EPA). 2020. Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments CASRN 335-76-2 (PFDA), CASRN 375-95-1 (PFNA), CASRN 307-24-4 (PFHxA), CASRN 355-46-4 (PFHxS), CASRN 375-22-4 (PFBA): Supplemental Information - Appendix A EPA/635/R-20/131. Washington, DC: Integrated Risk Information System, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. October 2019 Updated February 2020 (in response to public comments). 1-203
[227] (ATSDR 2021)

Barutcu et al. (2024)[228] and George and Birnbaum (2024)[229] have shown through transcriptomic analyses that PFAS elicit divergent biological responses depending on chain length, functional group, and dose. These findings further challenge the scientific foundation for assuming additivity among structurally and functionally distinct compounds.

b. The HI Framework Applies Additivity Without Scientific Justification or a Validated Common Mechanism

In vitro and animal studies cited by Birnbaum et al. (2025, pg. 36-37) such as those involving liver spheroids or mouse models, are often presented as evidence of dose-additive responses (REFs). For example, Birnbaum et al. (2025) note that "… human liver spheroids exposed to mixtures containing PFOA, PFNA, PFBS, PFHxS and/or PFOS were shown to change the expression of genes in a dose-additive manner[230]".[231] However, others have cautioned that such high-dose mechanistic studies are typically conducted at concentrations far exceeding environmental relevance. Moreover, they often do not include clinical endpoints or reproduce full toxicological pathways observed in humans.[232,233] As such, while informative for

[228] Barutcu AR, Black MB, Andersen ME. 2024. Transcriptomic re-analyses of human hepatocyte spheroids treated with PFAS reveals chain length and dose-dependent modes of action. Toxicology and Applied Pharmacology. 489: 117013.
[229] (George and Birnbaum 2024)
[230] Addicks GC, Rowan-Carroll A, Reardon AJF, Leingartner K, Williams A, Meier MJ, Moffat I, Carrier R, Lorusso L, Wetmore BA, Yauk CL, Atlas E. 2023. Per- and polyfluoroalkyl substances (PFAS) in mixtures show additive effects on transcriptomic points of departure in human liver spheroids. Toxicological Sciences. 194(1): 38-52.
[231] (Birnbaum et al. 2025, pg. 37)
[232] (Barutcu et al. 2024)
[233] (Clewell 2024)

identifying potential interaction points, these studies cannot by themselves justify a cumulative hazard-based regulatory standard.

Others have also emphasized that dose addition should not be assumed in the absence of empirical evidence for parallel dose-response relationships and toxicological similarity. Anderson et al. (2022) concluded that the existing PFAS literature does not provide sufficient data to support the application of dose-additivity models to most PFAS, -- particularly for GenX and PFBS, (which differ substantially in both pharmacokinetics and observed health effects compared to legacy compounds like PFOA and PFOS.)[234]

B. EPA's Use of the HI for PFAS Departs from Its Own Precedent and Lacks Empirical Basis for Mixture Regulation

Birnbaum et al. (2025) suggest that "[g]rouping hazardous chemicals together as a 'mixture' is therefore an approach available to the US EPA under numerous laws".[235] While it is true that cumulative assessments are utilized under various statutes (e.g., the Clean Air Act and Superfund), those applications are typically limited to chemicals with well-established, shared MOAs, and a strong empirical foundation in human health outcomes. Others have argued that PFAS differ in structure, kinetics, and biological activity to such an extent that a general mixture

[234] (Anderson et al. 2022)
[235] (Birnbaum et al. 2025, pg. 39)

model lacks validity. Ritscher et al. (2018)[236], Cousins et al. (2020)[237], and Anderson et al. (2022)[238] all emphasize that these differences prevent the application of generalized grouping strategies without a validated framework.

Even researchers advocating for stronger PFAS regulation have acknowledged this scientific complexity. George and Birnbaum (2024)[239], while supportive of precautionary approaches, acknowledged that mixture modeling remains speculative for PFAS due to insufficient understanding of shared pathways. They suggest that methods such as relative potency factors (RPFs) may offer future promise, but current data limitations preclude robust application[240].

Finally, others have noted that real-world epidemiological evidence has not demonstrated cumulative health effects from PFAS mixtures at environmentally relevant concentrations. To date, no study has linked co-exposure to PFHxS, PFNA, PFBS, and GenX with additive or synergistic adverse outcomes. As Meek (2013)[241]

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[236] Ritscher A, Wang Z, Scheringer M, Boucher JM, Ahrens L, Berger U, Bintein S, Bopp SK, Borg D, Buser AM, Cousins I, DeWitt J, Fletcher T, Green C, Herzke D, Higgins C, Huang J, Hung H, Knepper T, Lau CS, Leinala E, Lindstrom AB, Liu J, Miller M, Ohno K, Perkola N, Shi Y, Smastuen Haug L, Trier X, Valsecchi S, van der Jagt K, Vierke L. 2018. Zurich Statement on Future Actions on Per- and Polyfluoroalkyl Substances (PFASs). Environmental Health Perspectives. 126(8): 1-5.

[237] Cousins IT, DeWitt JC, Gluge J, Goldenman G, Herzke D, Lohmann R, Miller M, Ng CA, Scheringer M, Vierke L, Wang Z. 2020. Strategies for Grouping Per- and Polyfluoralkyl Substances (PFAS) to Protect Human and Environmental Health. Environmental Science Processes & Impacts. 22: 1-17.

[238] (Anderson et al. 2022)

[239] A.J. George, L.S. Birnbaum Dioxins vs. PFAS: science and policy challenges Environ. Health Perspect., 132 (8) (2024), Article 85003, 85003-4; 85003-5

[240] A.J. George, L.S. Birnbaum Dioxins vs. PFAS: science and policy challenges Environ. Health Perspect., 132 (8) (2024), Article 85003, 85003-5

[241] Meek MB. 2013. International experience in addressing combined exposures: increasing the efficiency of assessment. Toxicology. 313(2-3): 185-189.

and Pohl et al. (2024)[242] emphasize, mixture risk assessments must be grounded in human-relevant evidence and mechanistically plausible interactions, criteria that have not yet been satisfied for the PFAS compounds regulated under the current HI framework.

While Birnbaum et al. (2025) presented the HI as a reasonable and precautionary means of addressing PFAS mixture exposure, others have proposed that the approach lacks the scientific foundation necessary for enforceable regulation.[243] The regulated compounds differ in structure, toxicokinetics, and toxicodynamics, there is no established common MOA or dose-response pattern to justify dose additivity. The HI approach, as currently applied, reflects precautionary policymaking rather than a risk-based framework grounded in the best available science. Someday, a valid mixtures approach may be available, as we have for the dioxins and PCBs; but such data are not available for PFAS. There is not even a plausible mechanism for grouping the 35 PFAS commonly measured in drinking water. Under the Safe Drinking Water Act, enforceable standards must be supported by consistent, biologically relevant evidence, a threshold that current mixture models may not meet.

[242] Pohl HR, Buser MC, Ruiz P, Mumtaz MM. 2024. Mixtures Risk Assessment: Available Methods and Future Directions. In Paustenbach DJ, Farland W, Greim H, Klaunig J, Levy L. Patty's Toxicology, Seventh Edition. Hoboken, NJ, John Wiley & Sons Inc. 1-33.
[243] (Birnbaum et al. 2025)

Conclusion

The EPA's final regulation establishing national drinking water standards for select PFAS compounds represents a landmark in environmental health policy. However, other experts have expressed concern that the regulation may not be adequately grounded in the best available science, as required by the Safe Drinking Water Act. While the Agency's intention to protect public health is acknowledged and commendable, we believe the scientific foundation for several key regulatory decisions, particularly the derivation of MCLs and RfDs, warrants closer scrutiny.

The EPA's reliance on statistically significant but clinically modest associations in human epidemiological studies, coupled with high-dose animal bioassays and speculative mechanistic interpretations, has been questioned by a number of scientific reviewers. These associations, linking PFAS to outcomes such as increased cholesterol, reduced birth weight, liver enzyme elevations, and immunological changes, often fall within normal physiological ranges, lack robust dose-response relationships, and are confounded by other variables. Moreover, they have not been consistently linked to clinically adverse outcomes or elevated disease incidence.

We acknowledge that Birnbaum et al. (2025) strongly support the EPA's approach and emphasize precautionary principles.[244] However, their brief appears to

[244] (Birnbaum et al. 2025)

understate the uncertainty and variability in the underlying scientific data. Notably, key regulatory endpoints such as immunotoxicity, hepatotoxicity, and developmental toxicity, central to the EPA's risk characterization, were not substantively addressed in their brief, which others have interpreted as a recognition of the evidentiary limitations for those endpoints.

In addition, the continued reliance on rodent models that operate through PPARα-mediated mechanisms, despite longstanding recognition of their limited applicability to human health, raises further concerns about the biological relevance of the supporting data. While Birnbaum et al. (2025) cite mechanistic studies involving humanized mice and *in vitro* models, others have noted that these models are often exposed to supraphysiologic doses and do not replicate human complexity or real-world exposures.[245] Mechanistic plausibility, while informative, does not substitute for causal evidence at environmentally relevant exposure levels.

The use of the HI framework for regulating PFAS mixtures likewise reflects a precautionary policy decision rather than a mechanistically grounded risk assessment. Although Birnbaum et al. (2025) argue that additive effects justify this grouping, others have pointed out that the scientific literature does not support dose-response behavior.[246] This lack of empirical support for mixture effects has led many

[245] (Birnbaum et al. 2025)
[246] *Id*

to question whether the HI approach meets the statutory requirement for using the best available science.

In summary, while we respect the view expressed in the amicus brief by Birnbaum et al. (2025) and recognize the motivation to protect public health, we believe that the regulation must be based on scientifically robust, transparent, and human-relevant data.[247] Under the Safe Drinking Water Act, regulatory standards must be defensible not only in intent but also in execution, grounded in causal evidence, reflective of real-world exposures, and responsible to the evolving body of scientific literature. Where such standards are not met, the resulting rules may have unintended consequences for public trust, resource allocation, and long-term regulatory credibility.

For these reasons, and as laid out in the preceding sections, we respectfully submit that the Court should consider vacating the Final Rule on the grounds that it is arbitrary and capricious and not supported by the best available science.

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Respectfully submitted,
/s/Robert F. Redmond, Jr.

[247] *Id*

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